Louisiana State University, Department of Environmental Sciences
Response & Chemical Assessment Team

STANDARD OPERATING PROCEDURE FOR OIL SPILL SOURCE IDENTIFICATION

1. SCOPE

1.1 The following procedure details the extraction methodologies used to determine the concentration of oil analytes of interest in extracts prepared from many types of matrices for oil spill source identification. The oil analytes of interest are given in Table 1.

1.2 This method can be used to quantitate certain classes of organic compounds that are soluble in methylene chloride or hexane and are capable of being eluted, without derivatization, as sharp peaks from a gas chromatograph fused-silica capillary column coated with a slightly polar silicone.

1.3 The extraction procedures are different for each of the sample matrices; however, the instrumental analysis and report generation procedures are the same, regardless of matrix.

1.4 The internal standard mixture referred to in each extraction procedure is naphthalene-d_8, acenaphthene-d_{10}, chrysene-d_{12}, and perylene-d_{12} (usually at a concentration of 10 ng/uL). The surrogate standard mixture referred to in each extraction procedure is 5-alpha androstane and phenanthrene-d_{10}.

1.5 Good laboratory practices are utilized for each of the extraction procedures. This procedure does not address safety concerns associated with its use. It is the responsibility of the user to determine and execute proper safety and health practices. Use of this method should be restricted to trained and experienced personnel.

2. REFERENCE DOCUMENTS

2.1 ASTM Standards

D 5739-00 Standard Practice for Oil Spill Source Identification by Gas Chromatography and Positive Ion Electron Impact Low Resolution Mass Spectrometry

Comment [DAK1]: Doesn't require DFTPP tune to verify instrument autotune

2.2 EPA Methods

SW-846 3510C Separatory Funnel Liquid-Liquid Extraction
SW-846 3550B Ultrasonic Extraction
SW-846 3611B Alumina Column Cleanup and Separation of Petroleum Wastes
SW-846 3630C Silica Gel Cleanup
SW-846 8000B Determinative Chromatographic Separations
SW-846 8270D Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

Comment [DAK2]: Requires DFTPP tune

Table 1. Oil Analytes of Interest

Anthracene	**Fluoranthene**	**Phenanthrene**
Benzo (a) Anthracene	**Fluorene**	C-1 Phenanthrene
Benzo (a) Pyrene	C-1 Fluorene	C-2 Phenanthrene
Benzo (b) Fluorene	C-2 Fluorene	C-3 Phenanthrene
Benzo (e) Pyrene	C-3 Fluorene	C-4 Phenanthrene
Benzo (g,h,i) Perylene	**Hopanes**	**Pyrene**
Benzo (k) Fluorene	**Indeno (1,2,3-cd) Pyrene**	C-1 Pyrene
Chrysene	**Naphthalene**	C-2 Pyrene
C-1 Chrysene	C-1 Naphthalene	C-3 Pyrene
C-2 Chrysene	C-2 Naphthalene	C-4 Pyrene
C-3 Chrysene	C-3 Naphthalene	**Saturate Hydrocarbons**
C-4 Chrysene	C-4 Naphthalene	(nC_{10}-nC_{35})
Dibenzo (a,h) Anthracene	**Naphthobenzothiophene**	**Steranes**
Dibenzothiophene	C-1 Naphthobenzothiophene	**Triterpanes**
C-1 Dibenzothiophene	C-2 Naphthobenzothiophene	
C-2 Dibenzothiophene	C-3 Naphthobenzothiophene	
C-3 Dibenzothiophene	**Perylene**	

3. SIGNIFICANCE AND USE

3.1 The methodology provided herein is used for general qualitative oil characterizations and quantitative analysis of oil analytes of interest.

3.2 This SOP can also be used for assessing if spilled oil samples are a match or non-match to a source oil based on oil biomarkers that are source-specific chemical compounds and relatively resistant to environmental degradation. Extracted ion chromatograms, or ion fingerprints, from a "spill" sample can be compared to the same ion fingerprints from the "source" oil and the match/non-match determination is made by a qualitative comparison. If further justification is required, the ion fingerprints can be used to calculate source fingerprint indexes (SFIs) for the "spill" and "source" sample. Match/non-match determinations using the SFIs are made by plotting the average of replicate analyses of each SFI in a histogram with standard deviation error bars displayed.

3.3 The instrumental analysis and data processing aspects of this SOP focus directly on the generation of data using a list of target compounds (listed in Table 1) applicable to petroleum oil identification and includes petrogenic and pyrogenic sources of polycyclic aromatic hydrocarbons (PAHs) as well as, straight chain alkanes in the range of nC_{10} - nC_{35}.

3.4 This SOP provides a means of analyzing oils from light fuel oils through and including heavy fuel oils.

4. APPARATUS

4.1 Gas chromatograph interfaced to a mass spectrometer with a 70-eV electron impact ionization source. The system also includes a computer to control the instrumentation and an auto sampler for consistent injection of samples into the instrument system.

4.2 Capillary GC column, low bleed, fused silica, 5% diphenyl/95% dimethyl polysiloxane (e.g. J&W DB-5ms), 30 meters long, 0.25 inner diameter, and 0.25 micron film thickness.

4.3 Computer with data processing software for extracting oil fingerprints and for quantitative analysis.

5. REAGENTS AND STANDARDS

5.1 Only pesticide/reagent grade solvents will be used in all analyses and dish washing procedures.

5.2 Standards

5.2.1 *Calibration Standards*
A commercially-prepared oil analysis standard, available through Absolute Standards, is used to prepare the five-point calibration standards. Calibration standard solutions are stored in amber colored vials with PTFE-lined caps. The calibration standards are checked frequently for signs of degradation or evaporation and are replaced if the quality control check sample indicates a problem. In some applications, the calibration standard may contain additional analytes not included in the standard oil analysis mix.

5.2.2 *Internal Standard Solutions*
The internal standards are typically naphthalene-d_8, acenaphthene-d_{10}, chrysene-d_{10}, and perylene-d_{12}. The internal standards are bought and stored individually until they are mixed to make the internal standard solution. In some applications, the internal standard mix may contain additional standards not included in the typical internal standard mix.

5.2.3 *Reference Oil Standard*
The usual laboratory reference oil is Alaska North Slope Crude Oil (ANSCO). The ANSCO standard is prepared by extracting 1 gram of pure oil in 40-mL of solvent. The laboratory reference oil is analyzed in each sample batch as an additional QA/QC sample (a laboratory control sample).

5.2.4 *Surrogate Standards*
The surrogate standards are typically 5-alpha androstane (alkanes) and phenanthrene-d_{10} (aromatics). The surrogate standards are purchased and stored individually until they are mixed to make the needed concentration of surrogate standard. In some applications, the surrogate standard may contain additional standards.

6. QUALITY CONTROL

6.1 The GC/MS must be tuned using PFTBA to meet the recommended manufacturer criteria prior to the initial calibration and for each 12-hour period during which analyses are performed. Since LSU/DES-RCAT specializes in developing non-routine analytical approaches to study environmental pollution, it is our policy to establish specific quality assurance/quality control (QA/QC) plans on a project by project basis. Standard EPA methodologies and QA/QC guidance are not always applicable since they do not directly address the analysis of oil or the analysis of complex sample matrixes such as highly organic marsh sediments, and are mainly associated with analysis of analytes on EPA's "Target Compound" lists. Therefore, LSU/DES RCAT does not routinely run DFTPP tune for PAHs unless specifically requested by client.

Comment [DAK3]: LSU followed their SOP

6.2 A five-point calibration curve is performed quarterly. A continuing calibration standard (one point of the initial five-point calibration standard) is analyzed in each batch of samples or each 12-hour period during which analyses are performed. The acceptance criterion for the continuing calibration standard is ±20% of the average relative response factor calculated from the initial five-point curve. If the acceptance criterion is not met, all analyses are stopped until the instrument is performing at optimum conditions. Any instrument maintenance or troubleshooting may require a new five-point calibration curve to be performed.

6.3 If surrogate standards are used, extraction efficiency for each sample is evaluated based on the percent recovery of the surrogate standard. The acceptable percent recovery range is 70 – 120%.

6.4 LSU-RCAT has a separate QA/QC document which outlines other necessary procedures for ensuring data quality and is available upon request.

7. PROCEDURES

7.1 *Pure Oil Extraction Procedure*

Pure oil samples are usually extracted in a weight to volume manner. A ratio of 1 gram of oil to 40-mL of solvent (some extracts may be 0.25 grams oil in 10-mL of solvent or 0.50 grams oil in 20-mL solvent). Solvents commonly used include high purity hexane and dichloromethane (DCM). The pure oil sample is usually transferred into the extraction vial with a disposable pipette, if possible, or with clean, solvent-rinsed stainless steel spatulas. The weight of the oil sample is recorded and the solvent is then added. The vial is capped and then shaken to dissolve the oil in the solvent. At this point, it may be necessary to add pre-cleaned, granular anhydrous sodium sulfate to absorb any water from the extract. The vial with the extract is then placed in an ultrasonic bath for 15 minutes to settle out any particulates or asphaltenes. One milliliter of the extract is then transferred with a clean graduated, gas-tight syringe into an autosampler vial. If a dilution of the extract is necessary, the appropriate volume of extract to solvent ratio to achieve the correct dilution factor is

added to the autosampler vial with a graduated, gas-tight syringe. Internal standard is added, then the vial is capped and ready for analysis.

7.2 *Sediment/Soil Extraction Procedure*

Sediment/soil samples are homogenized by vigorous stirring then are sub-sampled for analysis. If the samples are frozen prior to sample extraction, the samples are transferred to a refrigerator until defrosted. For each sediment/soil sample, 30 g of material is accurately weighed (nearest 0.01g) into a pre-cleaned 500-mL beaker. Pre-cleaned, granular, anhydrous sodium sulfate is added and mixed into the sample until a "dry" sand-like matrix is created. One milliliter of surrogate standard is spiked into the sample and immediately, 100-mL of high purity DCM is added followed by approximately one minute of stirring with a spatula. The solvent level is marked to monitor any changes in the solvent volume. The beaker is then covered with two layers of aluminum foil and placed in a slightly warm, ultrasonic bath for 15 minutes. The warm solvent and vigorous sonication aid in enhancing extraction efficiency by ensuring intimate contact of the sample with the solvent. At the end of the first extraction series, the extract is allowed to settle for about one hour.

For grossly contaminated samples (high contamination) no further extraction is required and the extract is filtered through anhydrous, sodium sulfate and into a pre-cleaned jar. One milliliter of the extract is then transferred with a clean graduated, gas-tight syringe into an autosampler vial. If a dilution of the extract is necessary, the appropriate volume of extract to solvent ratio to achieve the correct dilution factor is added to the autosampler vial with a graduated, gas-tight syringe. Internal standard is added, then the vial is capped and ready for analysis.

For samples containing low to trace contamination, the extract is poured through pre-cleaned, granular, anhydrous sodium sulfate into a rotary evaporation flask. The extraction procedure is repeated two more times, each time with a fresh portion of DCM added to cover the sediment/soil sample in the beaker. During the last two extraction series, the sonication time is 15 minutes. Subsequent extracts are combined in a rotary evaporation flask and reduced to a final volume of 1-mL by a combination of rotary evaporation and "blowdown" under a gentle stream of purified nitrogen. The nitrogen blowdown is achieved by transferring the rotary evaporated extract with a disposable pipette into a graduated tube. The sample can then be further concentrated to 1-mL. The 1-mL of extract is then transferred with a clean graduated, gas-tight syringe into a 2-mL autosampler vial. Internal standard is added, then the vial is capped and ready for analysis.

If the sample results are to be calculated based on dry weight, a portion of the sediment/soil sample is prepared for drying in an oven overnight. Five to ten grams of sample are weighed in a pre-weighed aluminum weigh boat. The weigh boat with the sample is placed in an oven set for 105°C overnight. The sample is then removed and allowed to cool in a desiccator before determining the final, oven-dried weight of the sample. Percent dry weight is then calculated.

7.3 *Water Extraction Procedure*

Water samples are serially extracted with DCM in a separatory funnel. A measured volume of the water sample is quantitatively transferred to a clean separatory funnel with a PTFE stopcock in place and closed. One milliliter of surrogate standard is then added to the sample. If the entire water sample is used for the extraction, the original sample container is rinsed with 10 to 20-mL of DCM. If only a portion of the water sample is used for the extraction, the graduated cylinder used to measure the volume of the sample is rinsed with 10 to 20-mL of DCM. The DCM is poured into the separatory funnel containing the surrogate-spiked sample. The funnel is sealed and vigorously shaken and periodically vented for 1-2 minutes. The venting allows for the release of excess pressure in the funnel. The funnel is then placed on a ring stand to settle for a minimum of 10-minutes which allows the DCM to separate from the water. After the settling period, the DCM is drained from the bottom of the funnel, through a granular, anhydrous sodium sulfate filter, and into a rotary evaporator flask. The extraction procedure is repeated two more times using fresh portions of DCM. At the end of the extraction procedure, all three extracts are collected in the one rotary evaporator flask. Concentration of the final sample extract is achieved by rotary evaporation and nitrogen "blowdown". The nitrogen blowdown is achieved by transferring the rotary evaporated extract with a disposable pipette into a graduated tube. The sample can then be further concentrated to one to 10-mL. After concentration is complete, one milliliter of the extract is quantitatively transferred to a 2-mL autosampler vial and internal standard is added prior to capping the vial. The extract is now ready for GC/MS analysis.

7.4 *Tissue Extraction Procedure*

Individual bivalves in each sample are rinsed with distilled water thoroughly (including the shell and the tissue) to remove any material not associated with the tissue itself. If the bivalve samples are frozen prior to tissue extraction, the samples are transferred to a refrigerator until defrosted. All samples are shucked and the combined sample weight is recorded before homogenization. The organisms are then homogenized using a tissuemizer and stored in pre-cleaned jars. Approximately 5-10 grams of the homogenized tissue is removed from the sample and placed into a pre-cleaned 50-mL beaker. The weight of the homogenized tissue to be extracted will be determined by the actual quantity of bivalve specimens collected. Specimens from the same sampling site may require compositing to achieve the desired extraction weight. Previous DES/RCAT research has indicated no significant differences in the analyte recovery between the digestion and non-digestion methods; therefore, no digestion is performed. Granular, anhydrous sodium sulfate is added to the tissue in quantities of 15-25 grams depending upon the amount of water within the tissues or until a paste consistency is obtained. The sample is spiked with surrogate standard and then 35-mL of DCM is added to the paste. The beaker is covered with two layers of aluminum foil and sonicated for 15 minutes. After soniciation, the solvent extract is filtered through additional anhydrous sodium sulfate and pre-cleaned glass wool into a round bottom flask. The entire extraction procedure is repeated an additional two times with fresh aliquots of DCM.

To concentrate the solvent extract, the sample is rotary evaporated to approximately 2-mL final volume in DCM. The sample can then be split: 1-mL for lipid analysis; and 1-mL for GC/MS analysis. The GC/MS sample is solvent exchanged from DCM to hexane by adding 40-

mL of hexane to the 1-mL GC/MS fraction of the extract. The sample is concentrated again by rotary evaporation and nitrogen blowdown to 2-mL in hexane. The sample is fractionated on an alumina/silica gel column by placing the 2-mL hexane aliquot on the aluminum/silica gel column, which is then rinsed with high purity hexane. The flow of hexane is stopped prior to exposing the silica gel to air. This fraction contains the normal alkanes. The alumina/silica gel column is then rinsed with 50% DCM and 50% hexane. The solvents are allowed to elute completely. This fraction contains the PAHs. The alkane and PAH fractions are combined and concentrated to 0.l-mL under a gentle stream of nitrogen and stored until GC/MS analysis.

For the determination of dry lipid weight, the 1-mL lipid sub-sample is filtered through a clean, 0.1 micron filter into a clean, pre-weighed scintillation vial. The scintillation vial is then loosely covered and the solvent allowed to evaporate. The dry lipid weight is recorded and the final lipid weight calculated and reported.

8. INSTRUMENTAL ANALYSIS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY)

8.1 *GC Operation*

All GC/MS analyses use an Agilent 7890A GC system configured with a 5% diphenyl/95% dimethyl polysiloxane high resolution capillary column (30 meter, 0.25 mm ID, 0.25 micron film) directly interfaced to an Agilent 5975 inert XL MS detector system. An Agilent 7638B series Auto Injector is used for sample introduction into the GC/MS system. The GC flow rates are optimized to provide a required degree of separation, particularly n-C_{17} and pristane should be near baseline resolved, and n-C_{18} and phytane should be baseline resolved. The injection temperature is set at 250°C and only high-temperature, low thermal-bleed septa are used in the GC inlet. The GC is operated in the temperature program mode with an initial column temperature of 55°C for 3 minutes then increased to 280°C at a rate of 5°C/minute and held for 3 minutes. The oven is then heated from 280°C to 300°C at a rate of 1.5°C/min and held at 300°C for two minutes. Total run time is 66.33 minutes per sample. The interface to the MS is maintained at 280°C. Ultra High Purity (UHP) Helium is the carry gas for the GC/MS system.

8.2 *MS Operation*

The MS is operated in the Selective Ion Monitoring (SIM) to maximize the detection of several trace target constituents unique to crude oil. The instrument is operated such that the selected ions for each acquisition window are scanned at a rate greater than 1.4 scans/sec with a dwell time of 60 milli-seconds. At the start of each analysis period or every twelve hours, the MS is tuned to PFTBA, an internal instrument standard. Laboratory reference standards such as a reference oil and a continuing calibration standard are also analyzed prior to the analysis of the unknown sample extracts. This standard operating procedure ensures quality assurance/quality control of the instrument conditions prior to sample analysis.

8.3 *Quantitative Analysis*

Spectral data is processed by Chemstation™ Software using a customized data analysis method developed by DES. The analysis method is run on each sample and results in raw integration data that is transferred to a spreadsheet program for quantitative analysis. A macro printout is also generated and contains the extracted ion chromatography data in addition to raw integration data. Each macro printout is carefully reviewed and reintegrated as required.

Analyte concentrations are calculated based on the internal standard method. Therefore, an internal standard mixture, composed of naphthalene-d_8, acenaphthene-d_{10}, chrysene-d_{12}, and perylene-d_{12} (usually at a concentration of 10 ng/uL) is spiked into the sample extracts just prior to analysis.

The concentration of specific target oil analytes is determined by a 5-point calibration and internal standard method. Standards containing parent (non-alkylated) hydrocarbons are used in the calibration curve. Alkylated homologues are quantified using the response factor of the parent, and are therefore, only semiquantitative. This is the standard procedure since alkylated standards are not available.

8.3.1 *Calculations*

8.3.1.1 CONCENTRATION OF ANALYTES IN A SAMPLE:

$$\text{Conc (ng/mg or ng/mL)} = (A_x * I_s * V_t * DF * 1000) / (A_{is} * RRF * V_i * M \text{ or } V)$$

A_x = area of analyte
I_x = concentration of internal standard injected (ng)
V_t = final volume of the total extract (mL)
DF = dilution factor
A_{is} = area of internal standard
RRF = average relative response factor
V_i = volume injected (µL)
M or V = mass if solid (mg) or volume if liquid (mL)

8.3.1.2 RELATIVE RESPONSE FACTOR:

$$RRF = (A_x * C_{is}) / (A_{is} * C_x)$$

A_x = area of analyte in calibration standard
C_{is} = concentration of the internal standard
A_{is} = area of the internal standard
C_x = concentration of calibration standard

8.4 *Surrogate Corrections*
Recovery of all trace level samples is estimated using a two aromatic hydrocarbon surrogates: 5 alpha androstane and phenanthrene-d_{10}. Sediment samples are never

corrected for recovery, but a 70%-120% surrogate recovery acceptance criteria does apply. Tissue samples are corrected for recovery using the same two surrogate standards and similar surrogate recovery acceptance criteria (70%-120%).

9. REPORT GENERATION

9.1 Spectral data is processed by Agilent Chemstation™ Software using a customized data processing method and macro developed by DES. Each data file is carefully reviewed and re-integrated as needed after the initial processing. The macro prints a specified set of chromatograms that are used for qualitative comparison. The customized data processing method creates a custom report that contains the raw integration data which is then exported to a spreadsheet for quantitative analysis.

9.2 The concentrations of specific target alkanes and PAHs are determined by response factors that are calculated from commercially available internal and calibration standards. The internal standards used in all analyses are naphthalene-d8, acenaphthene-d10, chrysene-d12, and perylene-d12. The calibration standards are prepared at five different concentrations (5-point calibration curve) and contain saturate alkanes in the range of nC_{10} through nC_{35} and each parent aromatic hydrocarbon. The calibration curve results in response factors that are used to calculate the individual analyte concentrations in the samples. It is important to mention that the alkylated homologues in the extracted samples are quantified by response factors generated by the unalkylated parent (e.g. the response factor generated for naphthalene (C-0) is used to calculate the C-l through C-4 naphthalene homologues). Therefore, the results of the quantified alkylated homologues are only semi-quantitative since alkylated homologue standards are not available. Recovery and extraction efficiency of all trace level samples are estimated using two aromatic hydrocarbon surrogate standards: 5-alpha androstane for the alkanes and phenanthrene-d10 for the PAHs. Acceptable surrogate recoveries are in the range of 70%-120%.

9.3 Results for all analytical methods are reported as a function of volume, wet weight, or dry weight values depending on the circumstances and sample. The final results of the quantitative analysis are reported at two significant figures. The standard LSU-RCAT report usually includes a summary of the incident, a table containing a list of the samples included in the report, a conclusions section, and a selection of individual ion chromatograms, histogram plots generated from the quantitative analyses, and source fingerprinting results.

10. SOURCE FINGERPRINTING CORRELATIONS

10.1 Source-fingerprinting is an environmental forensics technique that utilizes analytical chemistry to compare samples of spilled oil to a suspected source to assess if the oil is a positive match. Since biomarker compounds are more resistant to environmental weathering processes, compared to most other oil compounds, they can be utilized as conserved reference compounds against which the loss of less stable oil components can be quantitatively estimated by calculating certain ratios. These ratios may be useful in differentiating unknown spill samples from a suspected source. Furthermore, the

distributions of oil biomarkers is unique for different types and blends of petroleum products and represent an oil-specific fingerprint to which distinct oil samples can be correlated. Match/nonmatch determinations can be achieved qualitatively through visual comparison of ion chromatogram patterns, and quantitatively from calculating the ratio of one biomarker to another. Ratios of certain biomarkers are referred to as source fingerprint indexes (SFI).

10.2 SFI are calculated by using the ratio of different peaks within the same isomer having similar retention times and identical mass to charge ratios. Choosing isomer groups that have similar water solubilities, vapor pressures, and parent masses will result in potentially useful SFI and contribute to the reduction of instrumental variance effects. As instrument conditions change because of matrix effects, column degradation, sensitivity, or tune degradation, both integers used to calculate the index (assuming they are similar in molecular weight, chemistry and quantitation ion) will be affected by the same relative degree of change; therefore, the index or ratio of the two integers, should remain constant. After a corrected base line value and peak heights have been determined, the SFI are calculated by dividing peak a by peak b in the isomer group. The goals of the SFI approach are to reduce investigator bias through the use of improved quantitative fingerprint techniques; and allow investigators to distinguish subtle differences in actual spill samples that can be easily missed by standard qualitative approaches.